

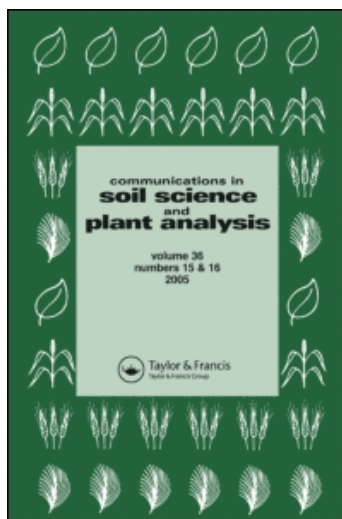
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### Can Near or Mid-Infrared Diffuse Reflectance Spectroscopy Be Used to Determine Soil Carbon Pools?

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POSTER PAPER

**Can Near or Mid-Infrared Diffuse  
Reflectance Spectroscopy Be Used  
to Determine Soil Carbon Pools?**

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**Abstract:** The objective of this study was to compare mid-infrared (MIR) and near-infrared (NIR) spectroscopy (MIRS and NIRS, respectively) not only to measure soil carbon content, but also to measure key soil organic C (SOC) fractions and the  $\delta^{13}\text{C}$  in a highly diverse set of soils while also assessing the feasibility of establishing regional diffuse reflectance calibrations for these fractions. Two hundred and thirty-seven soil samples were collected from 14 sites in 10 western states (CO, IA, MN, MO, MT, ND, NE, NM, OK, TX). Two subsets of these were examined for a variety of C measures by conventional assays and NIRS and MIRS. Biomass C and N, soil inorganic C (SIC), SOC, total C, identifiable plant material (IPM)

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(20× magnifying glass), the ratio of SOC to the silt + clay content, and total N were available for 185 samples. Mineral-associated C fraction,  $\delta^{13}\text{C}$  of the mineral associated C,  $\delta^{13}\text{C}$  of SOC, percentage C in the mineral-associated C fraction, particulate organic matter, and percentage C in the particulate organic matter were available for 114 samples. NIR spectra (64 co-added scans) from 400 to 2498 nm (10-nm resolution with data collected every 2 nm) were obtained using a rotating sample cup and an NIR-Systems model 6500 scanning monochromator. MIR diffuse reflectance spectra from 4000 to 400  $\text{cm}^{-1}$  (2500 to 25,000 nm) were obtained on non-KBr diluted samples using a custom-made sample transport and a Digilab FTS-60 Fourier transform spectrometer (4- $\text{cm}^{-1}$  resolution with 64 co-added scans). Partial least squares regression was used with a one-out cross validation to develop calibrations for the various analytes using NIR and MIR spectra. Results demonstrated that accurate calibrations for a wide variety of soil C measures, including measures of  $\delta^{13}\text{C}$ , are feasible using MIR spectra. Similar efforts using NIR spectra indicated that although NIR spectrometers may be capable of scanning larger amounts of samples, the results are generally not as good as achieved using MIR spectra.

**Keywords:** Carbon, soil, SOC, SIC,  $\delta^{13}\text{C}$

## INTRODUCTION

Previous research (McCarty et al. 2002) demonstrated that diffuse reflectance spectroscopic analysis in both the near-infrared (NIR, 400–2500 nm) and mid-infrared (MIR, 2500–25,000) ranges provide the means for measuring soil inorganic carbon (C) (SIC), soil organic C (SOC), and total soil C. McCarty et al. (2002) also observed that the presence of C as carbonates reduced the ability to measure SOC when comparing acidified vs. nonacidified samples. Importantly, the results with NIR spectroscopy (NIRS) and MIR spectroscopy (MIRS) across diverse soils collected over a large geographic region indicated that regional calibrations are feasible.

Although measurement of SIC, SOC, and total soil C are highly important, the ability to quantify various SOC pools and properties is also needed to aid in the determination of the source of the C and understanding SOC dynamics and C cycling. Computer models are increasingly used to assess and predict soil C stocks and turnover at local, regional, and national levels (Eve et al. 2001; Sperow, Eve, and Paustian 2003). The results obtained help satisfy policy-making needs and negotiation activities and potentially will be used as C markets develop. Process-based models such as Century (Parton, Stewart, and Cole 1987) include the input of plant materials and estimate the active, slow, and recalcitrant soil organic matter (SOM) fractions for prediction purposes. Organic matter is primarily C (about 58% by weight), with a large reservoir of essential plant nutrients contained in it and is also generally associated with the finer and more reactive clay and silt fractions of soils.

The input of plant-derived materials is the primary C source going into the SOC, and researchers make constant efforts to separate the plant- and

microbial-derived materials found in soils as described by Follett (2001). Once the plant material that is in contact with the soil becomes humified, intensive diagnostic procedures are required to measure the resulting C pools. The photosynthetic pathway of a majority of terrestrial plant species employs the  $C_3$  (Follett et al. 2001; Hoefs 2004), or Calvin, photosynthesis pathway that results in organic C that is approximately 18% depleted in  $^{13}C$  with respect to atmospheric  $CO_2$ . Carbon uptake by a minority (perhaps 20%) of terrestrial plant species is via the  $C_4$ , or Hatch–Slack, photosynthesis pathway with a carbon isotope fractionation that is only 6% depleted in  $^{13}C$  with respect to atmospheric  $CO_2$ . The  $C_4$  pathway is thought to be an adaptation to  $CO_2$ -limited photosynthesis, which developed relatively late in earth history and is advantageous under warm, dry, and/or saline environments (Hoefs 2004).

Alternative and efficient methods to measure several SOC fractions with a single instrument are needed to provide data inputs for models and other purposes because of current time-consuming, labor-intensive, and expensive analytical methods associated with measurements of individual SOC fractions. The objective of this study is to compare the abilities of MIRS and NIRS not only to measure SIC, SOC, and total C but also to measure key SOC fractions and the  $\delta^{13}C$  in a highly diverse set of soils while also assessing the feasibility of establishing regional diffuse reflectance calibrations for these fractions.

## MATERIALS AND METHODS

### Soil Collection and Conventional Analysis

Samples were collected from 14 sites in 10 western states (CO, IA, MN, MO, MT, ND, NE, NM, OK, TX) and comprised a very diverse set of soil samples (Follett et al. 2001). Further details on the nature of these samples may be found in Follett et al. (2001) and McCarty et al. (2002). Analyses for SOC fractions were conducted on samples that were delimed (acidified) as described by Follett and Pruessner (2001). A Carlo Erba C/N analyzer (Haake Buchler Instruments, Inc., Saddle Brook, NJ) was used to measure total C and N and the organic C and N contained in individual fractions. For SIC determination, a subsample of each sample was analyzed for total C as described previously with

$$SIC = \text{total C (nonacidified sample)} - \text{total C (acidified sample)}.$$

Of the original 237 samples, data for  $\delta^{13}C$  were available for 114, and data for other analytes such as total C were available for 185 (Table 1). The following analytes were determined for the two sample subsets: biomass C and biomass N were determined using the chloroform fumigation–incubation (CFI) method (Jenkinson and Powlson 1976) as described by Follett and Schimel

**Table 1.** Proximate composition of soil samples

Analyte	N	Mean	SD	Minimum	Maximum
Biomass, carbon, and nitrogen composition data					
Biomass C ( $\mu\text{g/g}$ soil)	185	528.42	643.65	3.56	4933.11
Biomass N ( $\mu\text{g/g}$ soil)	185	90.90	116.19	-126.11	812.85
Soil Inorganic C ( $\mu\text{g/g}$ soil)	185	0.36	0.64	-0.40	4.67
IPM (g/g soil)	185	0.0054	0.0093	0.0000	0.0504
SOC/SiltClay	185	0.025	0.025	0.001	0.144
Soil Organic C (%)	185	1.46	1.37	0.09	9.80
Total C (%)	185	1.82	1.52	0.12	10.39
Total N (%)	185	0.17	0.14	0.02	1.19
Samples with $\delta^{13}\text{C}$ data					
MCF (g/g soil)	114	0.64	0.22	0.11	0.96
MCF $\delta^{13}\text{C}$ (‰)	114	-19.12	3.28	-29.18	-13.48
POM $\delta^{13}\text{C}$ (‰)	114	-20.89	3.50	-27.48	-13.85
MCF C (%)	114	2.65	2.02	0.71	12.63
POM (g/g soil)	114	0.36	0.22	0.04	0.89
POM C (%)	114	2.29	2.90	0.10	15.52

IPM = identifiable plant material.

SOC/SiltClay = ratio of soil organic C to silt and clay content.

MCF = mineral-associated C fraction.

POM = particulate organic matter.

(1989). Biomass C and biomass N can provide information about the active SOM fraction for process-based models. Identifiable plant material (IPM) represents plant material that eventually becomes humified into SOM. Data for IPM represented plant material with a size of  $>2$  mm that could be separated from the soil samples by handpicking under a  $20\times$  magnifying glass. Because the larger IPM fraction was removed, measures using NIRS and/or MIRS represent IPM of a similar composition, but of a size  $<2$  mm that was not removed by handpicking.

The  $\delta^{13}\text{C}$  (Equation (1)) was determined by mass spectrometric analysis using a Carlo Erba C/N analyzer (Haake Buchler Instruments Inc., Saddle Brook, NJ) interfaced to a Tracer mass isotope-ratio mass spectrometer (Europa Scientific Ltd., Crewe, England). The  $\delta^{13}\text{C}$  sign and value indicate whether a sample has a higher or lower  $^{13}\text{C}/^{12}\text{C}$  isotope ratio than a calcium carbonate standard known as PDB from the Cretaceous Pee Dee formation in South Carolina (Boutton 1991).

$$\delta^{13}\text{C}(\text{‰}) = \frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}} - (^{13}\text{C}/^{12}\text{C})_{\text{reference}}}{(^{13}\text{C}/^{12}\text{C})_{\text{reference}}} \times 1000 \quad (1)$$

On the smaller subset of samples on which  $\delta^{13}\text{C}$  data were measured, the following analytes were determined: particulate organic matter (POM) was determined on soil that had been dispersed in a 0.5% hexametaphosphate and then was retained on a 53- $\mu\text{m}$  sieve after rinsing several times with deionized water. The fraction containing the mineral-associated C (MCF) was that part of the sample that passed through the 53- $\mu\text{m}$  sieve. The POM C (POMC) and MCF C (MCFC) were determined using a Carlo Erba C/N analyzer on the oven-dried samples ( $56^\circ\text{C}$ ) recovered from the sieving process. The POMC  $\delta^{13}\text{C}$  and MCFC  $\delta^{13}\text{C}$  were determined by mass spectrometric analyses. Further details on the exact methods used to determine each of these fractions may be found in Cambardella and Elliott (1992) and Paul, Morris, and Bohm (2001).

### Mid-infrared Spectroscopy

Samples were scanned in the MIR on a Digilab (Varian Instruments, Walnut Creek, CA) FTS-60 Fourier Transform spectrometer equipped with a KBr beam splitter and a DTGS (deuterated triglycine sulfate) detector. Samples were scanned in the wave number range of 4000 to 400  $\text{cm}^{-1}$  (2500 to 25,000 nm) at 4- $\text{cm}^{-1}$  resolution with 64 co-added scans per spectrum. Scanning was performed using ground but non-KBr diluted (Reeves 2003) samples using a custom-made diffuse reflectance accessory, which allowed a sample area of 50  $\times$  2 mm to be scanned for each sample (Reeves 1996). Further details can be found in McCarty et al. (2002). It should be noted that the sample cell was simply filled with soil and leveled with the top of the sample cell using a smooth edge, which has been shown to be sufficient for accurate quantitative analysis in many past studies.

### Near-infrared Spectroscopy

Samples were scanned in the NIR from 400 to 2498 nm on a NIRSystems model 6500 scanning monochromator (FOSS-NIRSystems, Inc., Laurel, MD) equipped with a rotating sample cup and PbS (1100 to 2498 nm) and Si (400 to 1098 nm) detectors. Data were collected at a nominal resolution of 10 nm with data collected every 2 nm with 64 co-added scans per spectrum. Although data were collected from 400 to 2498 nm (25,000 to 4000  $\text{cm}^{-1}$ ), only data from 1100 to 2498 nm (700 data points per spectrum) were used in the partial least squares analysis (PLS).

### Statistical Analysis

Summary statistics and correlation analysis were performed using SAS (SAS Institute, Cary, NC) PROC Means and PROC Corr version 8.

### Chemometrics

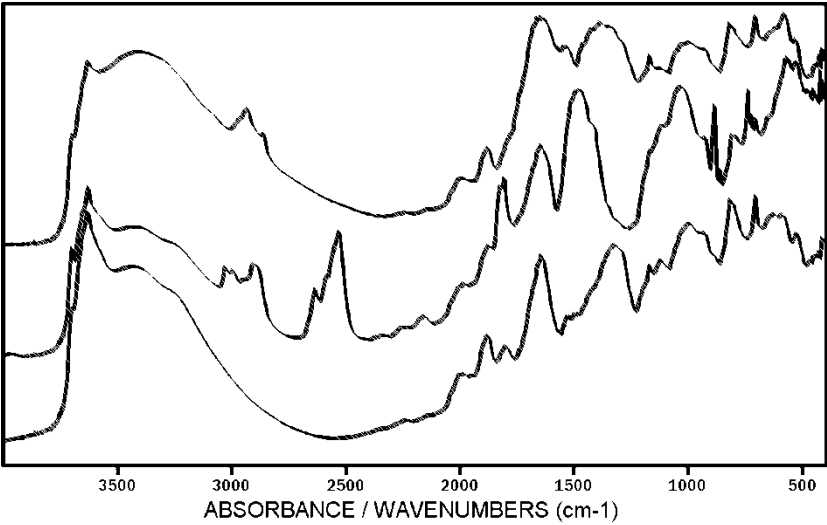
Calibration development was carried out using SAS PLS with a custom-made program (Reeves and Delwiche 2003). All spectra were mean centered and variance scaled. In addition, spectral pretreatments using first and second gap derivatives with gaps of 4 to 64 data points with and without multiplicative scatter correction (MSC) were tested. In all, 22 different spectral pretreatments were tested for all analytes. In addition, initial testing was performed using different spectral ranges and data point averaging to determine the optimal spectral range to use in the final PLS calibration development. Calibrations were developed using the one-out cross validation procedure with and without an independent test set. For the independent test sets, approximately 10% of the  $\delta^{13}\text{C}$  (14 samples out of 114) and 20% (35 samples out of 185) were randomly chosen and set aside as independent test sets for the  $\delta^{13}\text{C}$  sample set and non- $\delta^{13}\text{C}$  sample sets respectively. For these efforts, the best calibration was determined using a one-out cross validation on the remaining 100 and 150 samples respectively and that calibration was then used to predict the values for the test set samples.

## RESULTS AND DISCUSSION

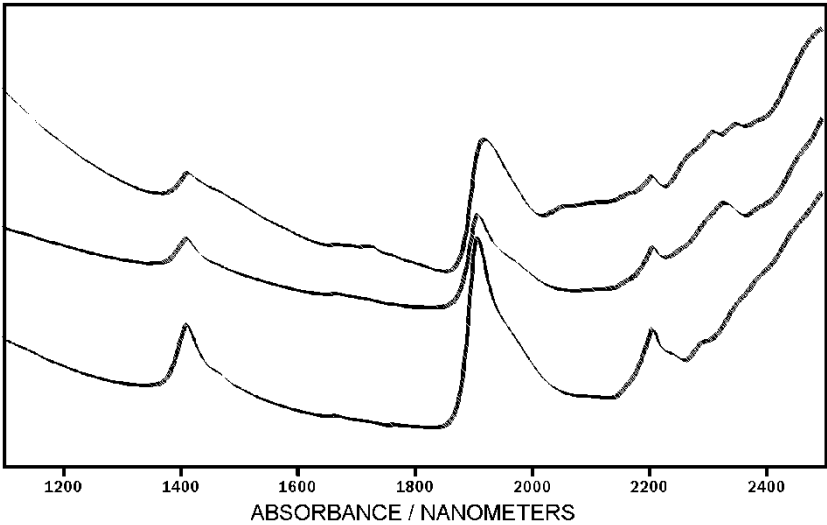
### MIR and NIR Spectra

In Figures 1 and 2, the MIR and NIR spectra of samples with the highest organic C, highest inorganic C, and a sample with relative low levels of both are presented respectively. As can be seen, the MIR spectra are always relatively more feature oriented than the NIR spectra. In the MIR, the presence of inorganic C or carbonates results in large peaks in the 2500 and 1500  $\text{cm}^{-1}$  region as demonstrated both by the differences between the three MIR spectra shown in Figure 1 and by their disappearance (data not presented) after sample acidification (McCarty et al. 2002). Although considerable spectral information is present because of the presence of organic C in the soil, as shown by comparing the high and low C spectra shown in Figure 1, the spectra also demonstrate that many MIR spectral bands in soils are due to noncarbonate mineral fractions present in the soil.

In comparison, the NIR spectra (Figure 2) show many fewer spectral features than the MIR spectra. The large bands at 1400 and 1900 nm are due to hydroxyl groups due almost entirely to residual moisture (all samples air dried) present in the sample. The band at about 2330 nm (center spectra) is due to carbonates as demonstrated by its disappearance with acidification (data not presented), whereas the other smaller bands before and after the 2350 band are due to various organic components present (McCarty et al. 2002). Although the NIR spectra may appear featureless, this does not mean that valuable information is not present. With proper data pretreatments



**Figure 1.** Mid-infrared spectrum of soil samples with highest organic C (9.80%, top spectrum), highest inorganic C (4.67%, middle spectrum), and low levels of both organic and inorganic C (0.09% and 0.03%, respectively, bottom spectrum).



**Figure 2.** Near-infrared spectrum of soil samples with highest organic C (9.80%, top spectrum), highest inorganic C (4.67%, middle spectrum), and low levels of both organic and inorganic C (0.09% and 0.03%, respectively, bottom spectrum).

(e.g., derivatives, etc.), numerous applications have demonstrated that sufficient information is present to develop accurate calibrations for a wide range of analytes in a wide range of materials (Williams and Norris 1987; Williams and Norris 2001) including soils (McCarty et al. 2002; Reeves, McCarty, and Meisenger 1999; McCarty and Reeves 2001).

### Sample Composition

Table 1 presents the compositional data used in this study. As shown, excepting the  $\delta^{13}\text{C}$  data, the analytes determined were all present in very wide ranges, which is good for calibration development. Note also that the soil samples varied greatly in composition as reflected by the wide geographical range from which they were obtained. Thus, samples with combinations of high total C, high organic C, and/or high inorganic C were present.

### Correlation Analysis

In Table 2, the results for correlation analysis of the available analytes are presented. Excluding correlations between components likely to be highly associated with one another (e.g., SOC with biomass N, TC and TN, etc.), the correlations were generally not very high. Thus, there does not appear to be any strong association between either of the  $\delta^{13}\text{C}$  measurements and the other measured analytes. The implication is that one could not predict either  $\delta^{13}\text{C}$  measurement from knowing the proximate composition of the sample in question.

### Calibrations Based on MIR Spectra

One-out cross validation results using MIR spectra are presented in Table 3. As can be seen, accurate calibrations for biomass; inorganic, organic, and total C (Figure 3); total N; MCF (Figure 4);  $\delta^{13}\text{C}$  MCF (Figure 5); MCFC; POM; and POMC were feasible. Results for other analytes varied in their accuracy but might still be useful as a quick method of determination. The type of accuracy expected can be seen by comparing the results shown in Figures 3–5 and also those achieved for  $\delta^{13}\text{C}$  POM ( $R^2 = 0.79$ , Figure 6). As can be seen (Figure 6) for analytes with  $R^2 < 0.80$ , considerable numbers of samples have spectroscopically determined values with an error greater than twice the RMSD, which is often quite large to start. Although such determinations might be useful in mass screenings, the degree of error for any particular sample can be large and must be kept in mind.

Table 2. Results of correlation analysis

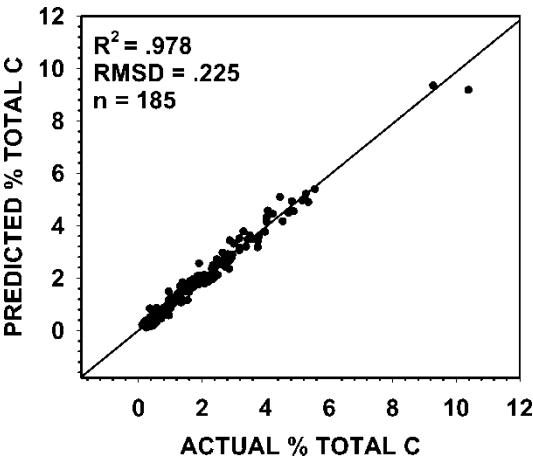
Analyte	MCF $\delta^{13}\text{C}$	POM $\delta^{13}\text{C}$	MCF C	POM	POM C	BIOM C	BIOM N	SIC	IPM	SOC/ SC	SOC	TC	TN
MCF	0.46	0.25	-0.54	-1.00	0.32	n.s. <sup>a</sup>	n.s.	n.s.	-0.29	-0.38	n.s.	n.s.	n.s.
MCF $\delta^{13}\text{C}$		0.87	-0.59	-0.46	n.s.	-0.24	-0.21	-0.52	-0.20	-0.45	-0.30	-0.43	-0.31
POM $\delta^{13}\text{C}$			-0.54	-0.26	n.s.	-0.28	-0.27	-0.43	-0.18	-0.41	-0.35	-0.44	-0.37
MCF C				0.54	n.s.	0.43	0.43	0.34	0.39	0.74	0.61	0.67	0.57
POM					-0.32	n.s.	n.s.	n.s.	0.28	0.37	n.s.	n.s.	n.s.
POM C						0.83	0.67	n.s.	0.56	0.48	0.75	0.69	0.77
BIOM C							0.89	n.s.	0.78	0.62	0.91	0.82	0.90
BIOM N								n.s.	0.68	0.57	0.80	0.72	0.76
SIC									n.s.	n.s.	n.s.	0.43	n.s.
IPM										0.55	0.66	0.56	0.61
SOC/SC											0.71	0.64	0.68
SOC												0.91	0.98
TC													0.90

<sup>a</sup>Not statistically significant  $p < 0.05$ .  
MCF = mineral-associated C fraction.  
POM = particulate organic matter.  
BIOM = biomass.  
SIC = soil inorganic C.  
IPM = identifiable plant material.  
SOC/SC = ratio of soil organic C to silt and clay content.  
SOC = soil organic C.  
TC = total C.  
TN = total N.

**Table 3.** Calibration results using mid-infrared diffuse reflectance spectroscopy

Analyte	MSC	Derivative	Gap	No. factors	Final calibration	
					R <sup>2</sup>	RMSD
Biomass C	Yes	First	4	13	0.935	163.5
Biomass N	Yes	First	4	4	0.649	68.7
Soil inorganic C	Yes	None	0	15	0.976	0.0992
IPM	No	Second	32	9	0.712	0.00497
SOC/SiltClay	Yes	Second	16	9	0.847	0.00965
Soil organic C	Yes	First	4	14	0.975	0.219
Total C	Yes	First	4	14	0.978	0.225
Total N	Yes	First	8	14	0.955	0.029
MCF	Yes	First	4	14	0.992	0.0195
MCF δ <sup>13</sup> C	No	First	16	12	0.918	0.933
POM δ <sup>13</sup> C	No	First	16	12	0.786	1.62
MCF C	Yes	Second	16	8	0.979	0.293
POM	Yes	First	4	14	0.992	0.0198
POM C	Yes	First	64	15	0.913	0.854

*Notes:* RMSD = root mean squared deviation of predicted–actual values.  
IPM = identifiable plant material.  
SOC/SiltClay = ratio of soil organic C to silt and clay content.  
MCF = mineral-associated C fraction.  
POM = particulate organic matter.



**Figure 3.** Final calibration results using mid-infrared spectra for total C.

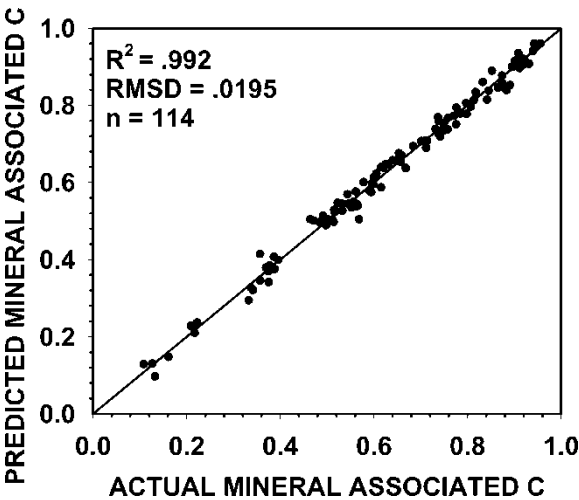


Figure 4. Final calibration results using mid-infrared spectra for percentage of C in the mineral-associated C fraction.

Calibrations Based on NIR Spectra

Results using NIR spectra for the same samples discussed previously are presented in Table 4. Comparing the NIR results to those achieved using MIR spectra (Table 3) shows that the NIR results were invariably poorer

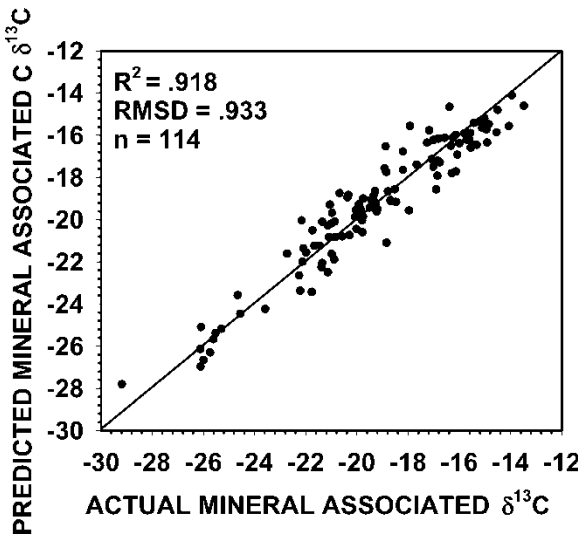
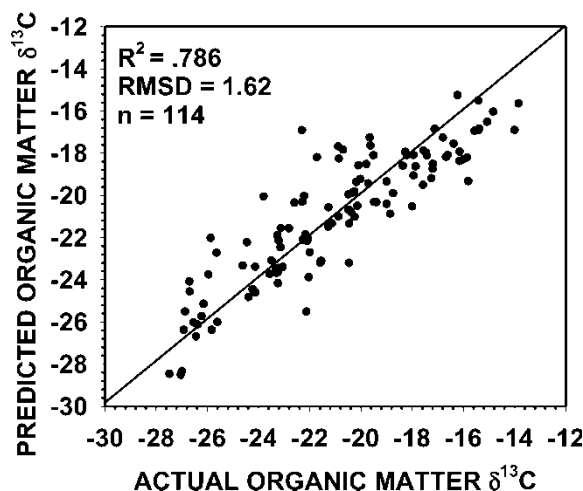


Figure 5. Final calibration results using mid-infrared spectra for mineral-associated C fraction  $\delta^{13}\text{C}$  values.



**Figure 6.** Final calibration results using mid-infrared spectra for particulate organic matter  $\delta^{13}\text{C}$  values.

(lower  $R^2$  and higher RMSD). Although the NIR spectrometer used in this study allowed a larger sample area to be scanned, this does not appear to be of any substantial benefit. Results for total C using NIR spectra can be seen in Figure 7. Comparing the results to those achieved using MIR spectra (Figure 3) shows no particularly bad samples (i.e., outliers), just an overall looser fit to the regression line. These results are similar to those found in previous studies for analysis of soil C content measures (McCarty et al. 2002). Although the NIR results for some analytes (e.g., inorganic, organic, and total C and others with  $R^2 \sim 0.9$  or greater) would still be very useful, for measures of  $\delta^{13}\text{C}$  in particular, the MIR would be much preferred.

### Results using an Independent Test Set

Although one-out cross validation results can provide some information on robustness of calibration, the use of an independent test set of samples, not involved in the calibration development, can be very useful in determining how the calibration might behave on future samples. In Tables 5 and 6, results are shown for calibrations developed using only 150 or 100 of the total sample set available. The remaining 35 and 14 samples (chosen randomly) constituted a validation or test set. As can be seen, results for the validation set were often, although not always (Figure 8, total C by MIR), considerably poorer than for the calibration set or full data set results (Tables 3 and 4). Again, the results for the NIR were generally poorer than

Table 4. Calibration results using near-infrared diffuse reflectance spectroscopy

Analyte	MSC	Derivative	Gap	Factors	Final calibration	
					R <sup>2</sup>	RMSD
Biomass C	No	Second	64	15	0.831	263.8
Biomass N	No	Second	16	7	0.617	71.7
Soil inorganic C	No	None	0	9	0.894	0.209
IPM	Yes	Second	64	10	0.689	0.00516
SOC/SiltClay	No	First	4	11	0.771	0.0118
Soil organic C	No	First	32	15	0.890	0.455
Total C	No	Second	64	15	0.903	0.473
Total N	Yes	Second	4	5	0.894	0.0448
MCF	Yes	First	64	14	0.939	0.0533
MCF δ <sup>13</sup> C	No	Second	32	5	0.748	1.64
POM δ <sup>13</sup> C	No	First	8	8	0.603	2.20
MCF C	Yes	Second	64	14	0.974	0.322
POM	Yes	First	64	14	0.936	0.0543
POM C	Yes	First	4	13	0.873	1.03

Notes: RMSD = root mean squared deviation of predicted–actual values.  
IPM = identifiable plant material.  
SOC/SiltClay = ratio of soil organic C to silt and clay content.  
MCF = mineral-associated C fraction.  
POM = particulate organic matter.

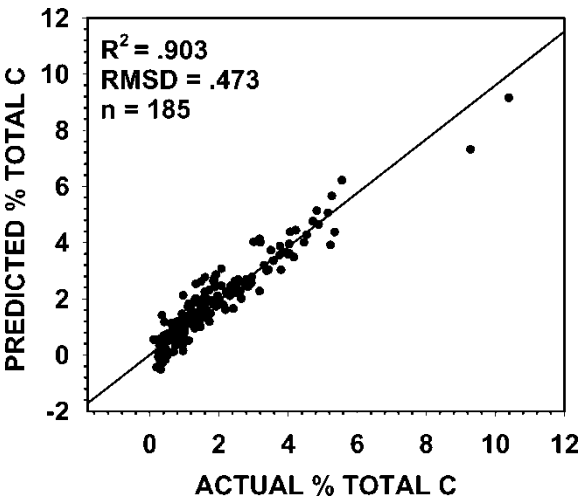


Figure 7. Final calibration results using nearinfrared spectra for total C.

**Table 5.** Validation results using mid-infrared diffuse reflectance spectroscopy

Analyte	MSC	Derivative	Gap	No. factors	Calibration		Validation	
					R <sup>2</sup>	RMSD	R <sup>2</sup>	RMSD
Biomass C	Yes	First	4	14	0.949	135.6	0.838	317.9
Biomass N	Yes	First	4	4	0.593	67.5	0.815	80.6
IPM	No	Second	8	6	0.690	0.00511	0.673	0.00554
Soil inor ganic C	No	Second	4	6	0.986	0.0755	0.939	0.180
SOC/ SiltClay	Yes	Second	32	12	0.880	0.00776	0.758	0.0164
Soil organic C	Yes	First	4	14	0.974	0.206	0.960	0.354
Total C	Yes	First	4	14	0.978	0.211	0.971	0.334
Total N	Yes	First	4	14	0.959	0.0273	0.929	0.0475
MCF	Yes	First	4	14	0.992	0.193	0.985	0.0300
MCF $\delta^{13}\text{C}$	No	First	8	12	0.928	0.852	0.715	2.07
POM $\delta^{13}\text{C}$	No	First	16	12	0.785	1.61	0.513	2.50
MCF C	Yes	Second	16	11	0.995	0.148	0.769	0.925
POM	No	Second	4	8	0.994	0.0166	0.967	0.0472
POM C	No	First	8	10	0.906	0.928	0.609	1.26

*Notes:* RMSD = root mean squared deviation of predicted–actual values.  
IPM = identifiable plant material.  
SOC/SiltClay = ratio of soil organic C to silt and clay content.  
MCF = mineral associated C fraction.  
POM = particulate organic matter.

**Table 6.** Validation results using near-infrared diffuse reflectance spectroscopy

Analyte	MSC	Derivative	Gap	No. factors	Calibration		Validation	
					R <sup>2</sup>	RMSD	R <sup>2</sup>	RMSD
Biomass C	Yes	Second	8	5	0.850	232.8	0.726	425.4
Biomass N	Yes	Second	4	3	0.552	70.8	0.687	98.2
Soil inorganic C	No	None	0	9	0.910	0.193	0.824	0.290
IPM	Yes	Second	64	10	0.676	0.00522	0.720	0.00518
SOC/SiltClay	No	First	4	13	0.816	0.00959	0.728	0.184
Soil organic C	Yes	Second	4	5	0.882	0.437	0.784	0.831
Total C	No	None	0	11	0.877	0.501	0.854	0.712
Total N	Yes	Second	4	5	0.897	0.0431	0.824	0.0633
MCF	Yes	None	0	15	0.932	0.0555	0.917	0.0841
MCF $\delta^{13}\text{C}$	No	Second	4	4	0.812	1.38	0.671	2.25
POM $\delta^{13}\text{C}$	No	Second	64	2	0.411	2.67	0.429	2.68
MCF C	Yes	Second	64	13	0.981	0.281	0.761	0.927
POM	No	None	0	12	0.896	0.0686	0.904	0.0775
POM C	Yes	Second	4	3	0.793	1.38	0.548	0.768

Notes: RMSD = root mean squared deviation of predicted–actual values.

IPM = identifiable plant material.

SOC/SiltClay = ratio of soil organic C to silt and clay content.

MCF = mineral associated C fraction.

POM = particulate organic matter.

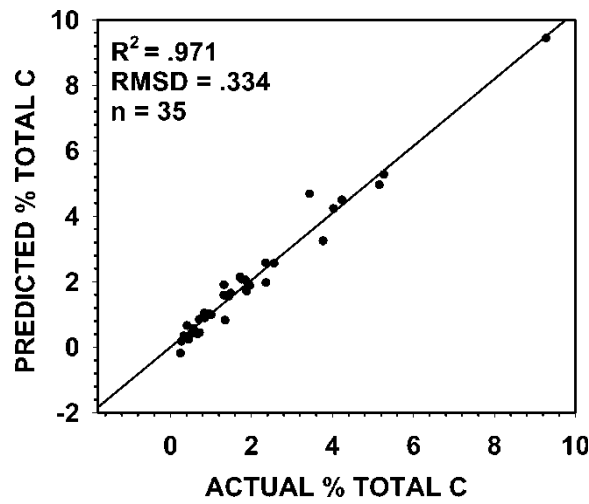


Figure 8. Validation set results for total C using mid-infrared spectra.

for the MIR with few validation results achieving  $R^2 > 0.9$  (MCF and POM only). The results for the MCF  $\delta^{13}C$  by MIR are shown in Figure 9.

Although not as good as might be desired, the results do indicate that accurate calibration for  $\delta^{13}C$  should be feasible with sufficient samples. It must be remembered that the samples in use were very diverse in nature and that 14 samples is a very small sample set, but the limited number of

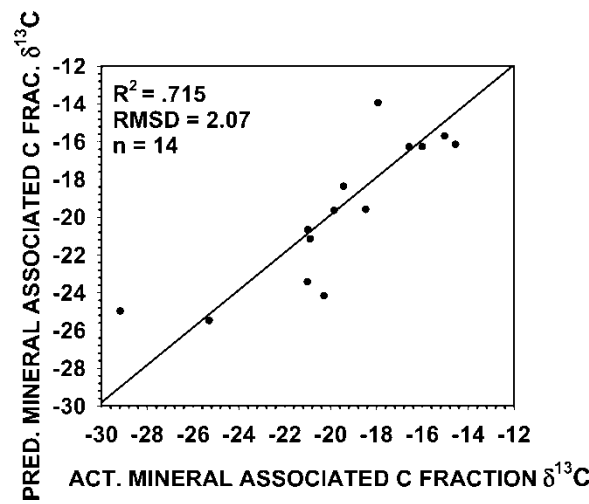
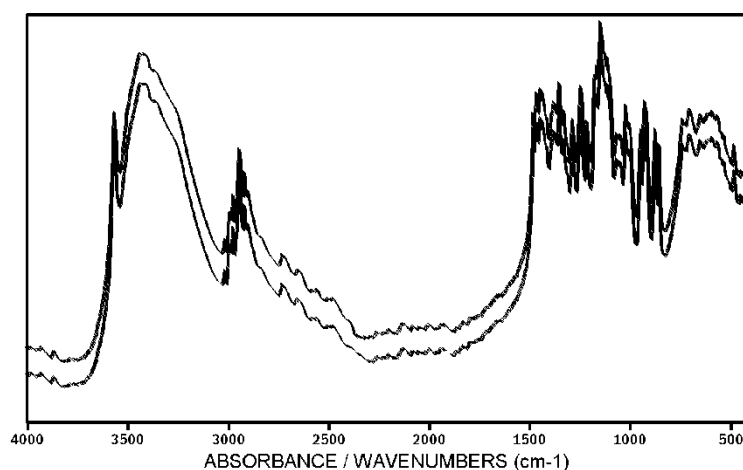


Figure 9. Validation set results for mineral-associated C fraction  $\delta^{13}C$  values using mid-infrared spectra.

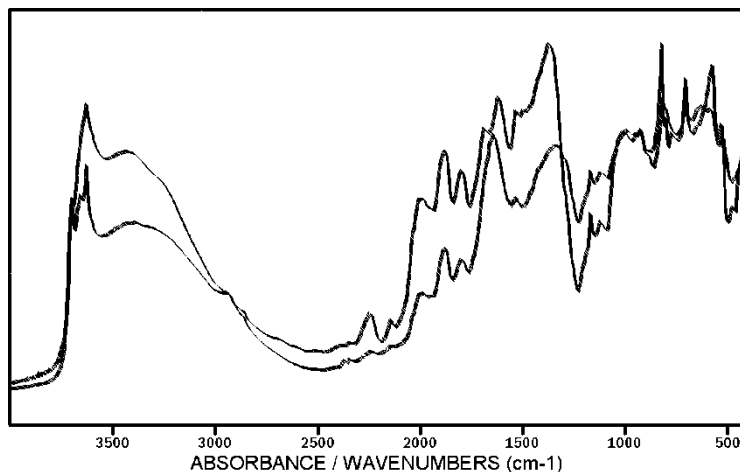
total samples available and the diverse nature of the sample set made using more samples unfeasible.

### Isotopic Spectral Differences

Although accurate calibrations for  $^{13}\text{C}$  isotopic differences appear to be feasible, the question of the spectroscopic basis for such calibrations is a difficult one to answer. As shown in Figure 10, the spectra of two sources of sucrose with different  $\delta^{13}\text{C}$  values of  $-11.45\%$  and  $-23.90\%$  ( $\pm .25$ ), a range comparable to that found in the soil samples (Table 1), are virtual traces of one another. Even expanded views of the two spectra (data not shown) show no peak shifts due to the difference in  $\delta^{13}\text{C}$  values. Examination of the literature indicates that in general such shifts are on the order of a few tenths of a centimeter $^{-1}$  and even then can be overwhelmed by shifts in bands due to temperature variations (Erdélyi, Richter, and Tittel 2002). The possibility of peak shifts being the basis for the calibrations cannot be ruled out, however. For example, it has been found that some bands due to aragonite in carbonates cause shifts of as much as  $18\text{ cm}^{-1}$  (White 1974). However, because of the many variations in composition occurring simultaneously, it is impossible to state that any of the spectral differences in the soil spectra examined here are due to  $\delta^{13}\text{C}$  differences even where the difference in the  $\delta^{13}\text{C}$  values is greatest as in the two samples shown in Figure 11. It is most likely that the spectroscopy is based on the same changes in C composition that the  $\delta^{13}\text{C}$  differences are used to determine or are based on. Thus, the  $\delta^{13}\text{C}$  differences are determined indirectly by what are generally labeled surrogate correlations.



**Figure 10.** Midinfrared spectrum of beet (top) and cane (bottom) sugars with  $\delta^{13}\text{C}$  levels of  $-23.9$  and  $-11.5$ , respectively.



**Figure 11.** Midinfrared spectrum of soil samples with highest (top) and lowest (bottom) MCF  $\delta^{13}\text{C}$  levels,  $-29.2$  and  $-13.5$ , respectively.

## CONCLUSIONS

Results using a diverse set of soil samples have demonstrated that accurate calibrations for a wide variety of soil C measures, including measures of  $\delta^{13}\text{C}$ , are feasible using MIR spectra. Similar efforts using NIR spectra indicated that although NIR spectrometers may be capable of scanning larger amounts of samples, the results are generally not as good as achieved using MIR spectra.

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